Claims

What is claimed is:

1. A method of isolating cells comprising,

(a) obtaining a tissue sample from a subject,

(b) successively exposing the tissue to a first solution with decreasing amounts of $CaCl_2$ comprising NaCl, HEPES, $MgCl_2$, KCl, and sugar at a pH of approximately 7.4,

(c) disassociating the tissue with an enzyme solution,

(d) repeatedly resuspending the disassociated tissue into a second solution with increasing amounts of $CaCl_2$ comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, and a fatty acid, at a pH of approximately 7.4 to obtain isolated cells.

2. The method of claim 1, further comprising the step of re-suspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid acid, and $CaCl_2$ at a pH of approximately 7.4.

3. The method of claim 1, further comprising the step of incubating the isolated cells in a mixture of carbon dioxide and air.

4. The method of claim 3, wherein the isolated cells are incubated at approximately 37°C.

5. The method of claim 1 wherein, the first solution is exposed to the tissue at approximately 37°C and at approximately 4 ml/min for 3 minutes.

6. The method of claim 1 wherein the concentration of $CaCl_2$ in the first solution decreases.

7. The method of claim 1 wherein the first solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

8. The method of claim 1 wherein the enzyme solution comprises a digestive enzyme.

9. The method of claim 8, wherein the digestive enzyme is a protease or a collagenase.

10. The method of claim 1 wherein the concentration of $CaCl_2$ in the second solution increases.

11. The method of claim 1 wherein the enzyme solution comprises approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM D-glucose.

12. The method of claim 1 wherein the second solution comprises Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, Ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1 μM at a pH of approximately 7.4.

13. A method of isolating cells comprising,

(a) obtaining a tissue sample from a subject,

(b) successively exposing at approximately 37°C the tissue to a first solution with decreasing amounts of $CaCl_2$ comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM sugar at a pH of approximately 7.4,

(c) disassociating the tissue with an enzyme solution for approximately 8 minutes comprising approximately 140 mM NaCl, approximately 10 mM HEPES, approximately 1 mM $MgCl_2$, approximately 5.4 mM KCl, and approximately 10 mM sugar, to form disassociated cells,

(d) repeatedly resuspending the disassociated cells into a second solution with increasing amounts of $CaCl_2$ comprising Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM at a pH of approximately 7.4 to form a solution of isolated cells,

(e) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C, and

(f) re-suspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and $CaCl_2$ at a pH of approximately 7.4 to obtain isolated cells .

14. A method of cultivating isolated cells comprising, resuspending the isolated cells approximately every 24 hours in a solution comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and $CaCl_2$ at a pH of approximately 7.4.

15. The method of claim 14 wherein the solution comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM, and approximately 1mM $CaCl_2$.

16. A cell culture media for cells comprising Earle's modified salt, L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES, fetal bovine serum, an antibiotic, a fatty acid, and $CaCl_2$ at a pH of approximately 7.4.

17. The cell culture media of claim 16 wherein the media comprises sodium bicarbonate at approximately 1250mg/l, creatine at approximately 328 mg/500ml, taurine at approximately 312 mg/500ml, ascorbic acid at approximately 8.8 mg/500 ml, HEPES at approximately 2.383 g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, a fatty acid at approximately 1 μM, and approximately 1mM $CaCl_2$.

18. A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and nitrilotriacetic acid;

(d) incubating the tissue in a second solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme;

(e) incubating the tissue in a third solution comprising calcium, salts, magnesium sulfate, pyruvate, glucose, taurine, HEPES, and a digestive enzyme; and

(f) centrifuging the tissue to obtain isolated cells .

19. The method of claim 18, further comprising the step of resuspending the isolated cells in a culture media comprising medium M199, BSA, ascorbic acid, taurine, carnitine, creatinine, insulin, and an antibiotic .

20. The method of claim 19, wherein the culture media further comprises a fatty acid or magnesium.

21. The method of claim 18, wherein the first solution comprises approximately 1-2 μM $CaCl_2$, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96.

22. The method of claim 18, wherein the second solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

23. The method of claim 18, wherein the third solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme.

24. A method of isolating cells comprising,

(a) obtaining a tissue sample comprising cells from a subject ;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2 μM $CaCl_2$, approximately 120mM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20,

approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

(e) bubbling approximately 100% O_2 through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM $CaCl_2$, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme; and

(h) centrifuging the tissue to obtain isolated cells.

25. A method of isolating and cultivating human myocardial cells comprising,

(a) obtaining a tissue sample comprising myocardial cells from a human subject;

(b) chopping the tissue;

(c) incubating the tissue in a first solution comprising approximately 1-2 μM calcium, approximately 120mM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and approximately 5 mM nitrilotriacetic acid, at a pH of approximately 6.96;

(d) shaking the tissue at approximately 37°C for approximately 12 minutes;

(e) bubbling approximately 100% O_2 through the solution;

(f) incubating the tissue in a second solution comprising approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20 mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 4 U/ml of a digestive enzyme;

(g) incubating the solution in a third solution comprising third solution comprises approximately 1-2 μM, approximately 30 μM NaCl, approximately 5.4 mM KCl 5.4, approximately 5 mM $MgSO_4$, approximately 5 mM pyruvate, approximately 20

mM glucose 20, approximately 20 mM taurine, approximately 10 mM HEPES, and 400U/ml of a digestive enzyme;

(h) centrifuging the tissue to obtain isolated cells;

(i) repeatedly resuspending the disassociated cells into a second solution which comprises increasing amounts of $CaCl_2$, Earle's modified salt, L-glutamine, sodium bicarbonate at approximately 1250mg/l, sodium pentothenate, creatine at approximately 328 mg/500ml, taurine at approximately 312mg/500ml, ascorbic acid at approximately 8.8 mg, HEPES at approximately 2.383g/500ml, fetal bovine serum at approximately 10% v/v, an antibiotic at approximately 5% v/v, and a fatty acid at approximately 1 μM at a pH of approximately 7.4 to form a solution of isolated cells; and

(j) incubating the isolated cells in a mixture of carbon dioxide and air at approximately 37°C.

26. A method of isolating and cultivating rodent myocardial cells comprising,

(a) removing the heart of a rodent;

(b) perfusing the heart with low calcium Tyrode's solution for approximately 3 minutes;

(c) perfusing the heart with an enzymatic solution for approximately 8 minutes;

(d) perfusing the heart with a low calcium solution for approximately 3 minutes;

(e) removing the ventricles;

(f) mincing the ventricles to isolate myocardial cells;

(g) mixing the cells in a low calcium solution;

(h) resuspending the cells in a solution comprising increasing concentrations of calcium; and

(i) resuspending the cells in culture media solution..